BioControl **48:** 561–578, 2003.

A distributed delay routine-based simulation model of *Beauveria bassiana* conidial stability in response to environmental stressors

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Received 20 June 2002; accepted in revised form 8 May 2003

Abstract. Using published data and equations on the relationship between spore longevity of the entomopathogenic hyphomycetes, *Metarhizium anisopliae* var. *acridum* and *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) and temperature and moisture content, a model of spore viability was constructed based on a distributed-delay routine. The model is modified via average spore survival time or by including an additional attrition (mortality) rate. The model was parameterized using published values from studies on *M. a.* var. *acridum* spores, and output compared favorably with germination data and with a previously-developed model. After initializing the model using parameter estimates of *B. bassiana* spores from the laboratory and published data on changes in (1) spore viability with respect to temperature and moisture content, and (2) spore moisture content with respect to temperature and relative humidity, the model was run using daily min/max temperature and relative humidity data and compared with data from four field experiments of Mycotech *B. bassiana* isolate GHA sprayed on canteloupe plants. For two of the experiments, observed viability trends were compared to model outputs using weather data from both a weather station and from within-canopy temperature and humidity probes. Output using weather station data fit observations much better than output using within-canopy probe data. For the two remaining sets of field data, both earlier in the season, only weather station data were available and the resulting output fit observations poorly. An attrition rate of 98% was needed to fit output to field data early in the growing season, and a rate of 74% was needed for data collected four weeks later. These attrition rates can be considered estimates for the proportion of spores dying for reasons other than temperature and relative humidity, and they were attributed largely to UVB radiation due to the more open canopy earlier in the season.

Key words: aerial conidia, *Beauveria bassiana*, distributed delay, simulation model, spore longevity

Introduction

Understanding the effects of abiotic factors on changes in spore viability over time is an important step in developing formulations of biopesticides

intended for industrial production (Jenkins and Grzywacz, 2000; Lacey et al., 2001). Simulation models can be useful tools in gaining that understanding, by providing a way of linking different kinds of observations to a common framework, and by helping to suggest new areas for experimentation. The shelf-life of spores in different regimes of temperature and relative humidity (RH) has been the subject of much research (e.g., Hedgecock et al., 1995; McClatchie et al., 1994; Moore et al., 1996), and is becoming more important as mycoinsecticides are becoming commercialized (Jaronski, 1997). Hong et al. (1997, 1998, 1999) developed and extended a model for the longevity of aerial conidia of *Metarhizium flavoviride* (Metschnikoff) Sorokin (now *M. anisopliae* var. *acridum* (Driver et al., 2000)) and *Beauveria bassiana* (Balsamo) Vuillemin (both Deuteromycota: Hyphomycetes). Hong et al. (1997) noted that most models of conidial longevity have assumed an exponential decay in germination rate over time (e.g., Meikle et al., 2001), with an exponential distribution of spore deaths. Hong et al. (1997) found that a better approach would be to assume a normal distribution of spore deaths over time, which produces a sigmoid decline in germination over time under most conditions.

Alternatively, one could consider the problem of modeling seed or spore viability using a distributed delay routine. Distributed delay routines (DDR) are used to model the aggregate flow of entities through a developmental phase and are incorporated frequently in simulation models (e.g., Gutierrez and Baumgaertner, 1984; Holst et al., 1997; Meikle et al., 1999; Larkin et al., 2000). Time-varying distributed-delay models can be driven by daily (or hourly or monthly) weather data, use a simple algorithm for determining the rate at which entities move through the process, and can include an attrition rate as a process that is separate from finishing the life stage.

In a typical age-structured population simulation model, the life of an insect, for example, would be divided into several life stages, e.g., egg, larva, pupa and adult. One life stage, such as an insect egg, fungus spore or plant seed, can be modeled using a DDR. Some life stages, such as the larva, will have two or more instars within the life stage, while others, such as the adult life stage, also include a process, reproduction, unique to that life stage. Life stages such as egg and pupa are more similar to spores in that they represent a (usually relatively resistant) state in which an organism remains for a certain period of time before either advancing to the next life stage or dying. The parameters that determine the rate of development, or aging, of an entity through such a life stage are usually estimated under various realistic regimes of temperature and humidity, regimes which include optimum and suboptimum conditions. Entities entering a DDR will exit the DDR distributed through time with an average delay (*DEL*) expressed in time units

(e.g., days) and variance $\sigma^2 = DEL^2/k$ specifying an Erlang distribution (Manetsch, 1976). Erlang distributions are gamma distributions for which the shape parameter k is an integer (Evans et al., 1993). At $k = 1$ (the lowest value) the Erlang distribution is an exponential distribution and as k approaches infinity it approaches the normal distribution (Manetsch, 1976), and a DDR can be specified for any distribution of spore deaths in that range. A time-varying DDR is one in which *DEL* is variable through time. For any DDR k will remain fixed. Larkin et al. (2000) provided a summary of a time-varying distributed delay routines.

The DDR as described above assumes that all entities pass completely through the process, although some individuals pass through faster than others and under conditions of high temperature or humidity the average transit time is lower. In this sense the modeled spores only exit the DDR because of "old age". A temperature of 45 °C, for example, might not kill spores outright but rather cause them to age at a rapid rate (at very high temperatures factors other than aging, such as the breakdown of enzyme systems, may become important but the model described here does not distinguish these factors). In addition to exiting the DDR due to "aging," as described above, Vansickle (1977) provided the basis for Manetch's (1976) DDR to account for entities leaving the process through disease, for example, or predation, UV light exposure, or any cause other than "aging." As Vansickle (1977) pointed out, the k stages of the DDR seldom have any biological meaning, and therefore a constant or time-varying attrition, or mortality, rate should be based on the entire DDR, rather than as, say, a Poisson process separately affecting each of the stages. In the Vansickle approach, the percentage dying due to an exogenous mortality factor was incorporated into the DDR spore model as an attrition rate that is proportional to the number of entities in the DDR at a given time that varies during the course of the DDR and that is a function of k, *DEL* and the known survivorship rate at the end of the experiment. The attrition rate at a given time is calculated on the level of the whole DDR process, and therefore, once a mortality rate is incorporated, *DEL* and the mean time through the process become different values than they would have with no attrition rate.

The objectives here were to (1) develop a DDR model of spore viability driven by temperature and either RH or moisture content; (2) evaluate the model output with initial parameters and laboratory data of spore longevity for *M. a.* var. *acridum*; (3) evaluate the model output with initial parameters, and field data of temperature, RH, and spore persistence, for *B. bassiana*; and (4) introduce an exogenous attrition rate, such as that caused by UV light exposure, in the model based on field observations of *B. bassiana* and explore the implications.

Materials and methods

Estimating the k *parameter of the distributed delay routine.* To estimate k, the *DEL* and the σ must be known. Hong et al. (1997) provided the following equation to calculate the germination rate (G) after p days:

$$\text{probit}(G) = \text{probit}(i) - p/\sigma \tag{1}$$

where i initial germination rate and σ is the standard error associated with the normally-distributed spore deaths over time. If p is considered *DEL*, that is, the number of days until the mean of the spore death distribution, then rearranging and solving for *DEL*:

$$DEL = (\text{probit}[i] - \text{probit}[G])\sigma \tag{2}$$

therefore the square root of the k parameter of the Erlang distribution can be estimated as probit(i) − probit(G). For normally distributed spore deaths over time with 100% initial germination rate, the mean would occur at $G = 0.5i$. In the case of an Erlang distribution, the mean would occur at $>0.5i$, the precise value depending on the particular k parameter and therefore not knowable in advance. The higher the k parameter, the less skewed the distribution and the closer the mean to $0.5i$. If the initial germination rate were about 95%, and we assume the mean to be about 50%, this would result in an estimate for k of about 1.64^2, or about 3 (only a rough estimate is needed, since the k parameter must be rounded to an integer).

The k parameter for the spore longevity model described here was estimated from published spore germination data. Hong et al. (1998) conducted a series of 14 experiments, using *M. a.* var. *acridum*, in which they monitored the germination rate of fungi kept at a constant temperature, 50 °C, and at moisture contents from 2.5 to 31.8%. *DEL* was estimated from the graphs themselves as the point at which the germination rate reached about 50%. The 50% germination rate was considered a reasonable estimate for the mean since, particularly in the case of Erlang distributions with low k parameters, the initial germination rate was always >90% and <100%. The variance was estimated using the equation:

$$\log_{10}\sigma = K - C_w \log_{10} m \tag{3}$$

where m is moisture content and K and C_w are fitted parameters and estimated as 3.662 and 3.059, respectively (Hong et al., 1998), for σ under a constant temperature.

Constructing a model of spore longevity. Hong et al. (1997) provided an equation for the standard deviation for the normal distribution of spore deaths over

time, using *M. a.* var. *acridum*, based on temperature in °C (d) and percentage moisture content (m):

$$\log_{10}\sigma = K_E - C_w \log_{10} m - C_H d - C_Q d^2 \tag{4}$$

where K_E, C_W, C_H and C_Q are fitted parameters. Given the k parameter estimated above, one can then use Hong et al.'s (1997) relation to express *DEL* as a function of the temperature and moisture content. Using this equation, a model was constructed which required the initial germination rate, the parameters of the Hong et al. (1997) equations relating viability to temperature and moisture content, and duration of modeling run (in days). The model is driven by a text file containing daily values of minimum temperature, maximum temperature, and moisture content. A time step of 1 hour was chosen to increase the precision of the DDR. The simulation model was programmed in C++, the implementation of the DDR closely following that of Abkin and Wolf (1976). Hong et al. (1999) provided daily germination data for *M. a.* var. *acridum* spores kept under a constant moisture content of 13.7% and 3 temperature regimes: 50 °C, 21 days at 30 °C followed by 50 °C, and 35 days at 30 °C followed by 50 °C. Output from the model, driven by those daily temperature and moisture content values, was compared to the Hong et al. (1999) data.

Model parameters for B. bassiana *field data.* Hong et al. (2001) examined seven different isolates of *B. bassiana*, found that the model structure (but not parameters) developed using *M. a.* var. *acridum* produced satisfactory results in the case of *B. bassiana*, and produced two sets of parameter values for their σ equation. Since the *B. bassiana* isolate used in our field experiment, Mycotech GHA (see below) was not among those tested by Hong et al. (2001), laboratory characterization of the viability of the Mycotech isolate under different temperatures was compared to output from the Hong et al. model using those two data sets to determine whether one set might be close enough to be considered an estimate of the Mycotech GHA isolate. To estimate moisture content of the spores in the field from the temperature and RH data, an equation developed by Hong et al. (2002), parameterized using spores of *B. bassiana* isolate JABB ID 9908-1330 (JABB of the Carolinas Inc., USA), was incorporated into the model program:

$$\begin{aligned} m \;=\; & 2.426 + 0.338r - 0.035d - 0.0071r^2 - 0.00081rd \\ & +0.000077r^3 + 0.000013r^2 d \end{aligned} \tag{5}$$

where m is moisture content, r is percentage relative humidity, and d is temperature in °C. We assumed that maximum and minimum temperatures occurred with the minimum and maximum values of RH, respectively. For

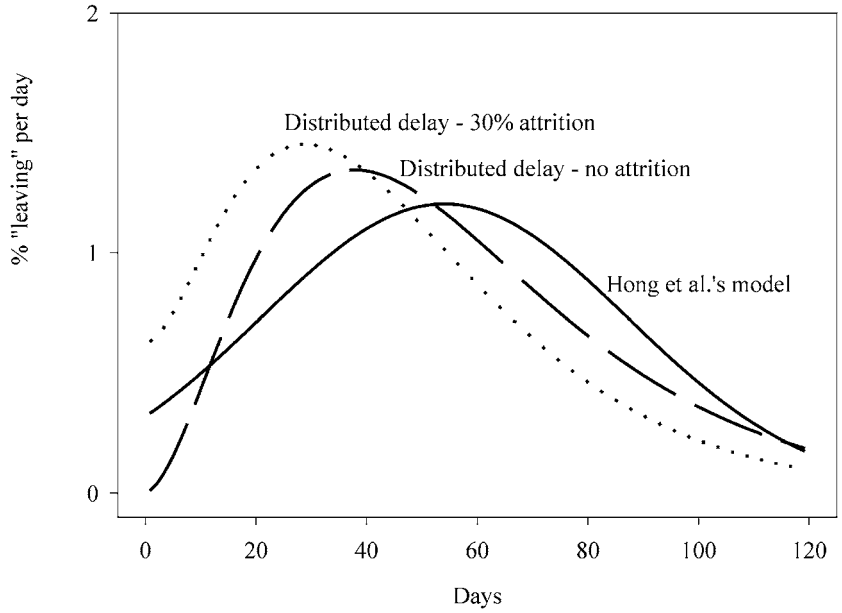

Figure 1. The distribution of spore deaths under different modelling regimes for spores kept at 35 °C and 12% moisture content with a 95% initial germination rate. The DDR model is shown with 100% survivorship (therefore comparable to the Hong et al. [1997] model of aerial conidia) and with 70% survivorship, to illustrate the change in the shape of the distribution.

each hour, the model interpolated temperature and RH values using a sine function (Allen, 1976), and then used equation [5] to estimate moisture content.

Attrition, that is, the loss of entities from the DDR for reasons other than aging, was specified in the model as the survival rate of entities entering the DDR. Attrition was assigned a value >0 only in some field data sets. The mean time that entities spend in the process decreases relative to *DEL* as the overall survivorship declines because entitities spending a longer time in the process will have been exposed longer to the probability of attrition and will therefore have a higher probability of being eliminated via attrition than do those that spend a shorter time (Vansickle, 1977). Elimination of the slower-developing entities has the effect of changing the shape of the percentage germination curve (Figure 1).

Measuring spore viability. Approximately 2 g of *B. bassiana* (Mycotech GHA) technical grade active ingredient and a similar amount of formulated wettable powder were stored in Nalgene vials in controlled temperature and humidity chambers. Moisture content was maintained at 6% and

temperatures were maintained at 25, 35 and 40 °C. Each week a sample was withdrawn from each vial and suspended in 3–5 ml of 0.04% Silwet L-77® (Loveland Industries Inc., Cambridge, UK). The suspension was vortexed at high speed for approximately 20 seconds and an aliquot streaked on Sabouraud dextrose agar + 0.1% yeast extract and 1 ml Gentocin®/L. (SDAY+). Inoculated media were incubated for 16–20 hours at 25 to 27 °C. At the end of this incubation a drop of 0.01% acid fuchsin in 85% lactic acid was placed on the agar surface with spores, covered with a coverslip and examined with 400X phase contrast microscopy. A spore was considered germinated if a hyphal peg was visibly emerging from it. At least 200 spores were counted in at least two to three fields.

Field data. Field data on the persistence of spores of *B. bassiana* (Mycotech GHA) were obtained for experiments conducted in Scottsdale AZ, in 1996 using Mycotrol WP® applied to commercial cantaloupe (*Cucumis melo* L. var. 'Topmark') (S.T. Jaronski, J.C. Lord and J. Rozinska, unpublished data). Mycotrol WP (at the time composed of conidia, attapulgite clay filler and amorphous silica desiccant) was suspended in 0.03% Silwet L77® at a rate of fungus equivalent to 1.12 kg WP (2×10^{13} conidia) in 465 l/ha. Conidial suspensions were applied to individual abaxial and adaxial leaf surfaces of test plants using a hand atomizer; applications thoroughly wetted leaf surfaces but were short of runoff. Fully expanded cantaloupe leaves of mature, flowering plants were used in these tests. Applications were generally made before 8:30 A.M. on the first day of the experiment. Subsequently, 3–4 leaves were chosen at random from among each treatment group at each sampling interval, generally daily, placed together in a plastic bag and immediately returned to the laboratory. The conidia were washed off the leaves by placing the leaves with 0.06–1.0% Silwet L77® in a plastic zip-lock bag and shaking vigorously for 1–2 minutes. Suspensions were plated on yeast-benylate agar (adapted from Milner et al., 1991) (0.5 g/l yeast extract, 16 g/l agar, 20 ml 1% Benomyl 50WP® (Dupont), 1 ml/l Gentocin®, 0.1g/l Tween 80®). This medium prevents hyphal elongation after spore germination, allowing more flexibility in incubation time. Conidia on the inoculated plates were examined, using the method described above for measuring spore viability, for germination after 20–24 hours of incubation at 24–28 °C. At least 200 conidia were examined for each sample. The field trial was conducted four times: May 15–24 (Julian Dates 136–145), June 21–July 1 (J. D. 173–183), July 5–14 (J. D. 187–196) and July 12–23 (J. D. 194–205), 1996.

Ambient temperature and relative humidity data were obtained from the local AZMET web site (http://ag.arizona.edu/azmet, data for "Scottsdale AZ" site) for all field experiments. Within-canopy temperatures and RH were also

monitored from 6 to 23 July, using probes linked to digital data recorders (HOBO® Onset Computer Corporation). The probe values were compared to those data obtained from the web site, and both sets of data were used to drive the model. Resulting model output was compared to field observations. Because probe data were unavailable earlier than 6 July, for earlier experiments the model was run using only the weather station data, and model behavior was further modified by including the attrition rate as described above.

Results and discussion

Estimating the k *parameter.* From the data provided in Hong et al. (1998, 1999), the average delay (time from start of an experiment to 50% of the initial viability) was estimated over a range of moisture contents. The respective k parameter estimates ranged from 1.4 (for moisture contents of 4.2% and lower) to 2.8, with most values for treatments with moisture contents above 4.2% falling between 2.0 and 2.8. Estimates using data from Moore et al. (1996) gave similar results, assuming moisture content to have been about 8%. The DDR model was initialized with a k parameter of 3 and, using equation parameters for *M. a.* var. *acridum* from Hong et al. (1999), was found to be acceptable with respect to the data from Figure 3 of that paper (Figure 2). It should be noted that, for simplicity, the parameters of the Hong et al. (1999) spore longevity model in Figure 2 are the same as those used for the DDR model and the same as those that were provided in that paper as characterizing the fungal isolate; the parameters are not the same as those that were used to generate the curves in original Figure 3 on p. 171 of Hong et al. (1999). The fit of the DDR model would be expected to be similar to that of the Hong et al. model because of the similarity in the shapes of the underlying distributions.

Parameterizing a model for B. bassiana. To construct a model of the Mycotech GHA isolate used in the field experiments, appropriate parameters for the Hong et al. (1997) σ equation were needed. Although these parameters have been estimated for several *B. bassiana* isolates (Hong et al., 2001), they have not been estimated for the Mycotech GHA isolate. Ignoring the initial viability values, parameters provided by Hong et al. (2001) fell into two main groups, differing in the K_E parameter (Table 1). The first group, referred to here as the I97-1119 Ascot group, were observed to have a relatively slower decline in viability over time across different temperatures and moisture contents compared to the second group, referred to here as the JABB isolate group. Both the DDR model and the Hong et al. model were

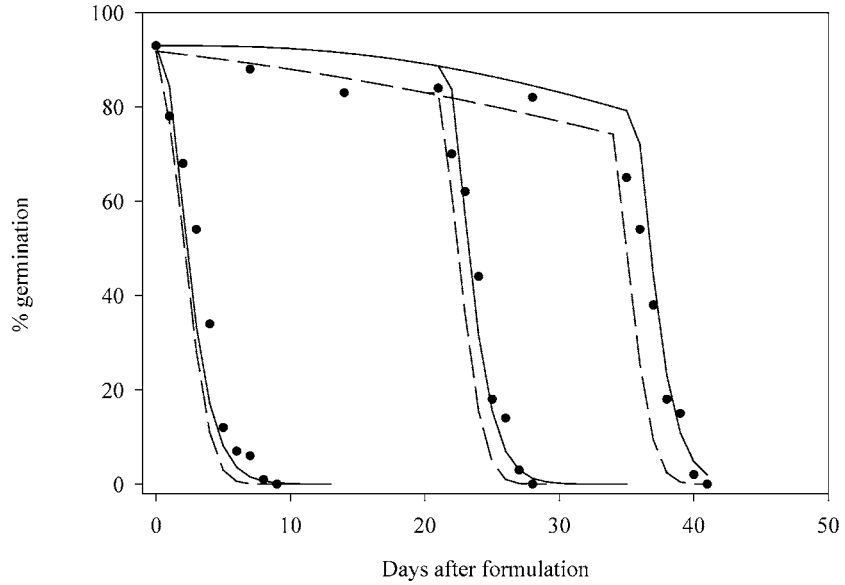

Figure 2. Comparison of model output using a distributed delay routine (solid line) and using the Hong et al. (1999) equation (dashed line) on data (solid circles) from Hong et al. (1999) (Figure 3, p. 171). The same parameters were used for both model outputs.

Table 1. Parameters for the spore viability equation [4] (from Hong et al., 2001)

Isolate	K_E	C_w	C_H	C_Q
I97-1119 Ascot	6.696	3.05	0.0293	0.00081
JABB	6.203	3.05	0.0293	0.00081

initialized using each set of parameters and model predictions for the number of days until 15% and 50% viability loss were compared to laboratory data for the Mycotech isolate at 25 °C, 35 °C and 40 °C, assuming a 95% initial viability and 6% moisture content (Table 2). The comparison shows that there are minor differences under the observed conditions between the two models, and that the JABB isolate group parameters provided a closer fit to the Mycotech GHA isolate than Ascot parameters. Accordingly, the JABB parameters were used to model the field data.

Field validation. Weather files were constructed using the temperature and RH data from the probes when possible, and using data available from the AZMET meteorological site for Scottsdale, Arizona. Between July 6 and

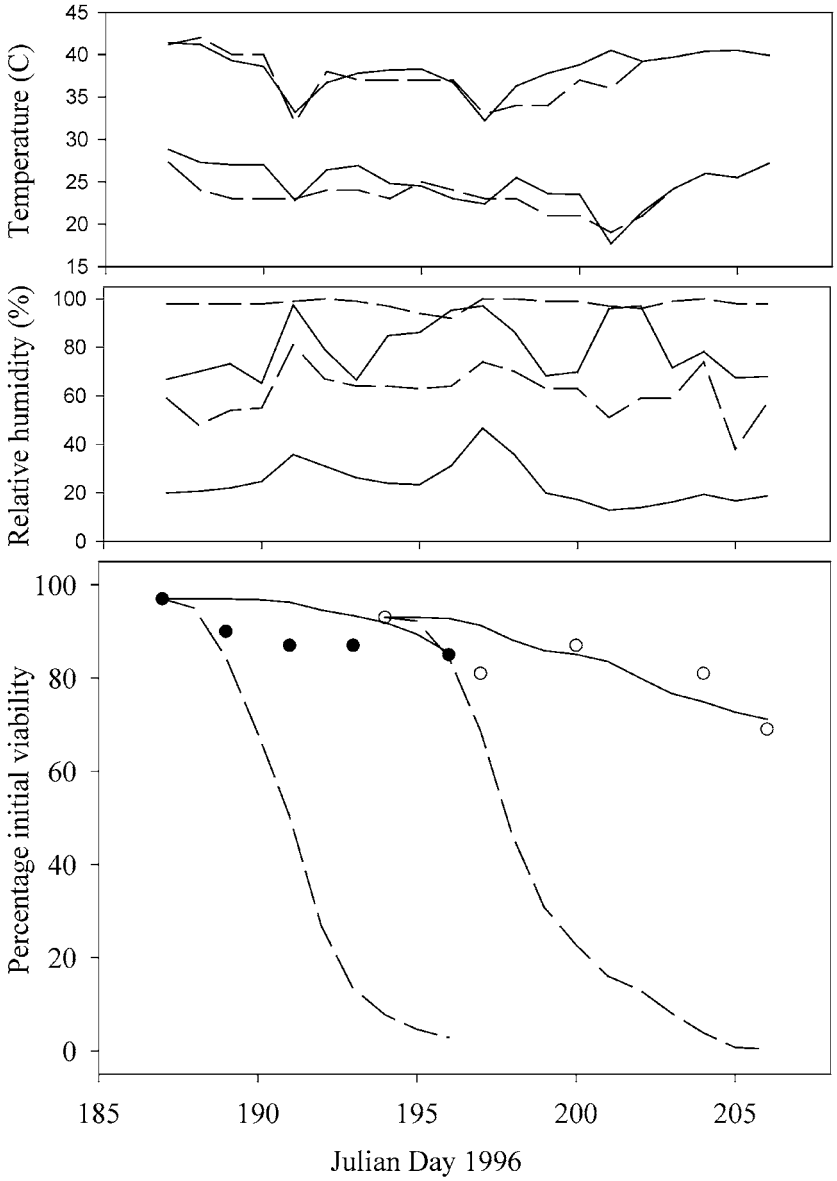

Figure 3. Comparison of distributed delay model output, bottom graph, with field data on *B. bassiana* conidia viability, collected in a melon field from July 5–14 (solid circles) and July 12–23 (empty circles), 1996, in Scottsdale AZ. The model outputs were generated using weather station data, upper two graphs (daily minimum and maximum values), from the web site http://ag.arizona.edu/azmet (solid line in all graphs) and probe data from within the plant canopy (dashed line in all graphs).

Table 2. Comparison of the number of days to losses of 15% and 50% viability, assuming 95% initial viability and 6% moisture content, for DDR model output using parameters from Hong et al. (2001) for two different *B. bassiana* isolates. "DDR model" refers to the model using a distributed delay routine, and "Hong model" refers to the model of spore longevity initially proposed by Hong et al. (1997)

	Mycotech		I97-1119 (Ascot)		JABB	
Temperature	Techn. grade active ingred.	Wettable powder	DDR model	Hong model	DDR model	Hong model
LT_{15} at 25 °C	167 (38–273)	247 (227–267)	729	744	234	239
LT_{15} at 35 °C	35 (0–95)	41 (11–87)	120	124	38	40
LT_{15} at 40 °C	11 (0–28)	16 (3–29)	42	44	13	14
LT_{50} at 25 °C	531 (196–725)	548 (506–590)	>1499	2002	533	643
LT_{50} at 35 °C	107 (9–219)	112 (78–194)	276	333	88	107
LT_{50} at 40 °C	44 (10–87)	47 (12–76)	97	118	31	38

July 19, when both probe and AZMET data were available, the two data sets were deemed sufficiently closely related to use one to estimate the other: the correlation coefficients (r) between the probe data and the AZMET were 0.72 for minimum temperature (significantly different from 0, d.f. = 15, $P < 0.003$) and 0.76 for maximum temperature (significantly different from 0, d.f. = 14, $P < 0.003$). AZMET minimum temperature values were, on average, 1.5 °C higher than the probe data, and AZMET maximum temperature values were, on average, 0.8 °C higher. Four minimum and five maximum probe temperature values were not available so AZMET data for those dates were used in the probe weather file. With respect to minimum RH, the correlation between probe and AZMET was $r = 0.77$ (significantly different from 0, d.f. = 14, $P < 0.003$), although probe data were, on average, 38% higher than weather-station measurements. Maximum RH values were not correlated at all ($r = -0.29$, not significantly different from 0). Probe values within the canopy were 97% or greater for 15 of the 17 days while AZMET data were less than 90% for all but four days. Two minimum RH probe data points were missing and were estimated in the probe weather file as AZMET data +38%. Two maximum RH probe data were missing and were estimated as 98%. The assumption (used as a basis for hourly interpolations of the weather file) that maximum and minimum temperatures were associated with the minimum and maximum RH values, respectively, was largely confirmed by the data.

Output from the model program run using probe weather data was compared to output from the program using weather station data for the two

experiments conducted in July when both sets of data were available (Figure 3). It should noted that, during this period, the melon leaves in the field were sufficiently developed to form a closed canopy above the soil, possibly providing protection to spores from UVB radiation (280–320 nm) (Jaronski et al., 1997). Many workers (e.g., Boulard et al., 2001; Pachepsky, 1999) have found significant differences between ambient temperature and RH, and temperature and RH as measured within the crop canopy or on the leaf surface, and indeed differences were noted here, particularly with respect to RH. However, the ambient weather data proved much better at predicting spore viability than did the probe data. Possible explanations are that (1) the model was incorrect: the estimated equation parameters were significantly in error, or other components of the wettable powder formulation or wetting agent interacted with spores, changing their behavior with respect to temperature, RH and/or moisture content, and that changed behavior was successfully modeled by chance by the ambient weather data; or (2) the probe data was incorrect: while the temperature probe apparently functioned well, the humidity probe either malfunctioned or succeeded in creating, and recording data from, its own micro-environment which did not reflect the conditions experienced by the spores, and the difference between ambient and canopy conditions was much less than indicated. Hong et al. (1998) suggested that the upper-moisture-content limit to their relation between conidia longevity and moisture content was probably similar to that determined for seeds by Roberts and Ellis (1989), that is, an equilibrium RH of about 90%. At equilibrium RH >90%, longevity of the spores would be expected to increase. This phenomenon, which would fall in the first of the two possible explanations listed above, would explain at least part of the lack of correspondence between the observed germination in the data sets in Figure 3 and the output from the model driven by probe weather data.

A comparison of model output, with no attrition (mortality) rate driven by weather station data, to field data from the first field trial in May showed a very poor fit (Figure 4, bottom graph). Indeed, while the observed spores lost over 40% of their viability in 9 days, under those weather conditions the spores were predicted to lose almost none (less than 1%) over that period. Some of this loss may be attributed to exposure to UVB radiation, which was not measured but was likely to have been greater earlier compared to later in the season since the plant canopy was more open and exposed earlier in the season. Exposure to UVB light has been observed to cause rapid mortality among many species of fungal spores (Fargues et al., 1996; Moore et al., 1996), although Braga et al. (2001) found the relationship somewhat more complex. Fargues et al. (1996) conducted a survey of the effects of artificial UVB irradiance on spore viability, as measured by the density of

colony-forming units, on 135 isolates of 4 entomopathogenic fungal species, including *B. bassiana* LRC 26, obtained from Mycotech. They found that after two watt-hours (W-hr) less than 1% of the spores were viable, and after four W-hr the density of viable spores was negligible. This suggests that even partly shaded spores would have a low probability of lasting two days. Alternatively, the loss may be due to interactions of spores with leaf exudates or phylloplane microbial flora, although why such interactions would be less important later in the season is unclear.

The model was modified, using an attrition rate over the entire DDR, to incorporate presumed losses to UVB for the first and second field trials (in May and June, respectively). The model was run using mortality rates from 1% to 99% at 1% intervals, and the output was quantitatively compared to the field data by calculating the sum of squared differences between model output and field data for each mortality rate. The mortality rate which produced the output with the least sum of squared differences was 98% in the case of the May field trial, in other words, although spores were observed for only nine days, to fit the model to the data, spore survivorship through the entire DDR was 2% (Figure 4). The corresponding mortality rate for the June trial was estimated to be 74% (Figure 5). In contrast, survivorship through the DDR for the field trials conducted in July, as described above, was 100%. One way to interpret this is that, given the validity of the weather data and the equation parameters and that spores died due to some mortality factor, 98% of the spores in the case of the first field trial would have eventually died due to that factor, compared to few or none later in the season. Although the reason for the lack of germination of a given spore is not known, this approach does provide a mortality rate, given a series of clearly stated assumptions, independent of temperature and humidity.

If the main assumptions of this approach are acceptable, that is, (1) the equations used do provide reasonable descriptions of the relationship of spore viability to temperature and moisture content and the relationship of spore moisture content to temperature and RH, and (2) the DDR approach is valid, two questions remained unanswered in the analysis: First, why did the ambient, rather than within-canopy, humidity data produce model output with the best fit to the field data? Second, did a mortality factor other than UVB exposure play a role in spore longevity in the field? The model output as described here relies on too many assumptions to be considered a refutation of the idea that the differences between weather station data and probe data are important, but it does suggest that further work is needed to understand the relationship between the field data (weather and spore viability data) and the model.

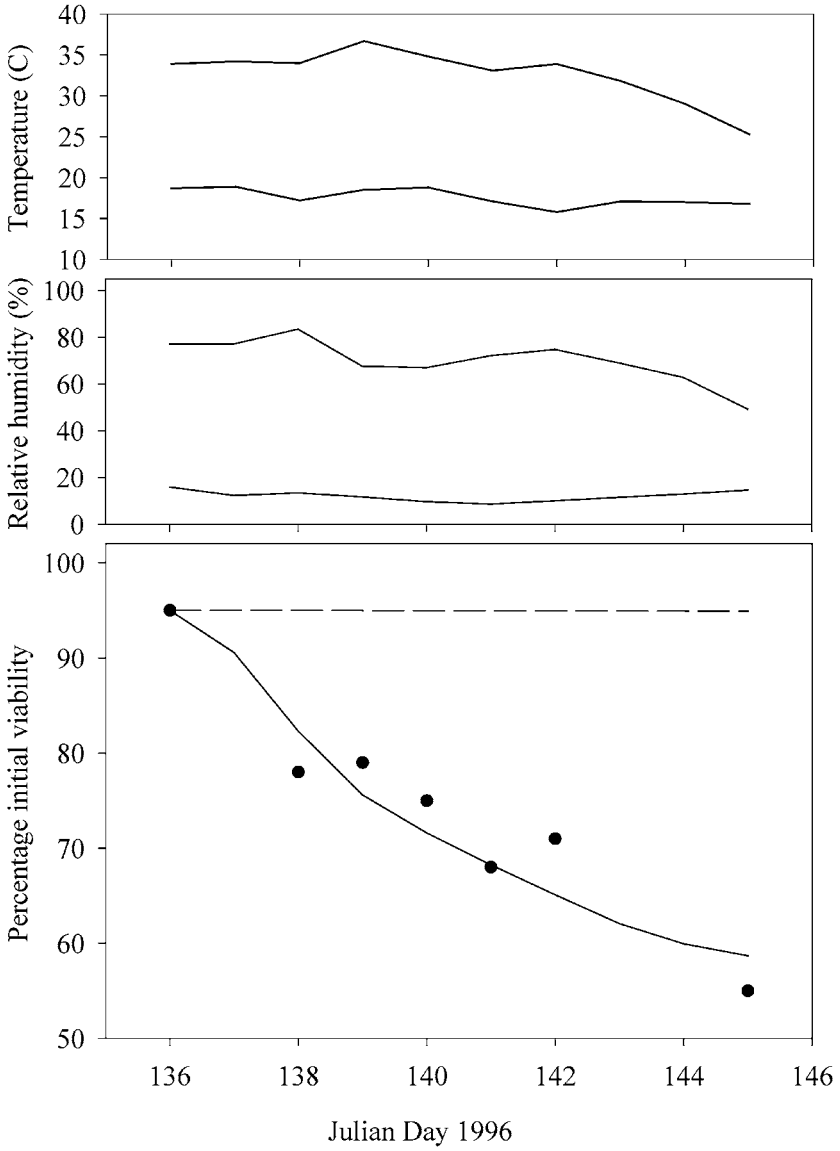

Figure 4. Comparison of distributed delay model output over time, bottom graph, under conditions of no attrition (dashed line) and 98% attrition rate (solid line) with field data (solid circles) on *B. bassiana* conidia viability, collected in a melon field from May 15–24, 1996, in Scottsdale AZ. The model outputs were generated using weather station data, (daily minimum and maximum values in each of the upper two graphs), from the web site http://ag.arizona.edu/azmet.

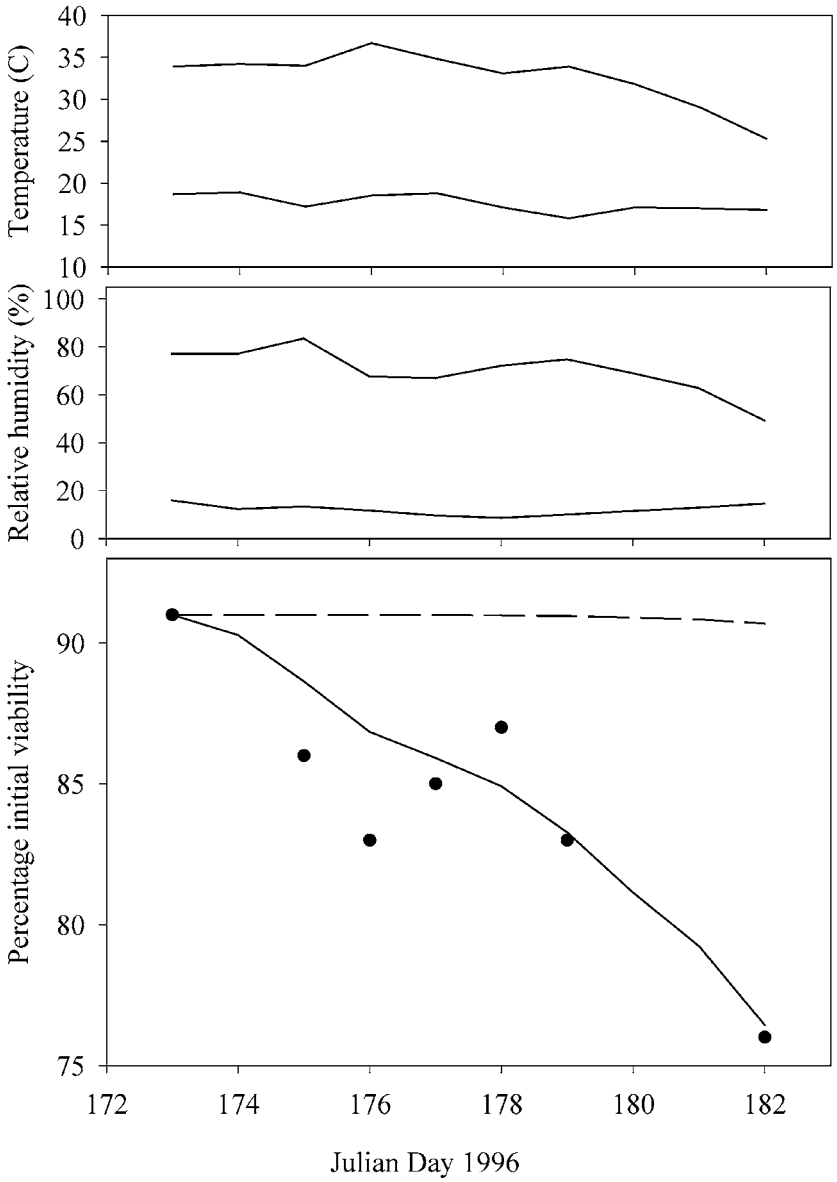

Figure 5. Comparison of distributed delay model output over time, bottom graph, under conditions of no attrition (dashed line) and 74% attrition rate (solid line) with field data (solid circles) on *B. bassiana* conidia viability, collected in a melon field from June 21–July 1, 1996, in Scottsdale AZ. The model outputs were generated using weather station data, (daily minimum and maximum values in each of the upper two graphs), from the web site http://ag.arizona.edu/ azmet.

The simulation model of spore viability presented here was easily constructed, but data sets needed for estimating the parameters exist for comparatively few fungi. Given satisfactory parameter estimates, models such as this can be used for a number of applications, such as predicting the shelf life of spores under different gas concentrations in packages of gas and moisture permeabilities. Using data on spore longevity under vacuum or under gases such as oxygen or nitrogen, the first step to model spore longevity would be to determine the relationship between spore longevity and temperature and moisture content under an optimal atmosphere regime, and then to modify that equation by measuring changes in germination rates over time under other atmosphere regimes. This model can also be linked to spatial data of, for example, temperature or light data, or to simulation models of pest dynamics to estimate the potential impact of a pathogen in a particular system.

Acknowledgements

We would like to thank J.C. Lord and S. Rosinska for help with field experiments, K. Hoelmer, N. Holst and T.G. Shanower for reviewing the manuscript, N. Jenkins for discussions and two anonymous reviewers for their very helpful comments.

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